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Conformational Ensembles in GPCR Activation

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Recent advances in G-protein-coupled receptor structural biology have provided only limited insight into the active conformations of these key signaling molecules. A paper from Nygaard et al. reveals the dynamic nature of GPCRs along the activation pathway by complementing NMR experiments with ultralong-timescale molecular dynamics simulations.

G-protein-coupled receptors (GPCRs)—numbering perhaps 900 in the human genome—represent the largest class of receptors in the human genome as well as the single largest class of targets for therapeutic drug discovery (Allen and Roth, 2011). We are currently in the midst of a "golden era of GPCR structural biology," with nearly 20 high-resolution GPCR structures having appeared since 2007 (Stevens et al., 2012) and several more likely to appear in coming months. These structures add immensely to our understanding of ligand binding to GPCRs; however, the majority represent inactive antagonist-bound conformations, and as a consequence, our understanding of the precise details responsible for agonist- and G-protein-initiated conformational changes is lacking. In this issue of *Cell*, the Kobilka and Shaw groups bring NMR and computational modeling to bear on the question of how β2-adrenergic receptors achieve their active conformation (Nygaard et al., 2013).

Despite the recent wealth of GPCR X-ray structures, we currently have only one structure of the elusive "ternary complex" (De Lean et al., 1980) containing receptor, agonist, and G protein (Rasmussen et al., 2011). Although crystal structures will provide invaluable information regarding the active state, they only represent a snapshot of the likely conformational states induced by agonist and G protein binding. Ultimately, it will take many distinct "frozen" receptor conformations to fully appreciate the conformational dynamics associated with GPCR activation. In the past 2 years, spectacular progress has been made on two fronts: NMR-based studies elucidating ligand-induced receptor dynamics (Liu et al., 2012) and long-timescale molecular dynamics simulations (Dror et al., 2011). Bringing these two approaches together, a team led by Brian Kobilka combined heteronuclear single-quantum coherence (HSQC) spectroscopy using C13 methionine with long-range molecular dynamics simulation to provide provocative new insights into the conformational dynamics associated with G protein and agonist binding to β2-adrenergic receptors (β_2ARs).

Because visualizing every residue by NMR to fully elucidate GPCR dynamics is beyond the scope of current technologies, Nygaard et al. instead sampled the chemical environment of five strategically located methionine residues in the βAR. They examined residues in regions of the receptor that are known to undergo dynamic changes and were able to identify distinct conformational states that are, perhaps, indicative of various GPCR states. The timescale in which apparent receptor motions occur has a critical role in determining the shape of the NMR peaks, and it was intriguing that the timescales and positions appeared to nicely complement the long-time molecular dynamics simulations performed independently. The molecular dynamics simulations require a special-purpose computer (a.k.a., "Anton") (Shaw et al., 2008) designed to accelerate classical MD simulations by orders of magnitude, allowing the authors to simulate conformational changes on much longer timescales than previously possible.

The proposed model resulting from this collaborative work suggests that both the cytoplasmic and extracellular halves of the receptor are highly dynamic (Figure 1). Upon agonist binding, the extracellular half is stabilized into an active-like conformation while the cytoplasmic half apparently becomes more mobile. Presumably, by thus exploring many structural conformations, the receptor has the potential to interact with multiple partners. Individual cofactors may then bind to and stabilize a different conformation. It is now clear that some ligands are "biased" in that they preferentially induce signaling through one pathway (e.g., G-protein-mediated signaling) versus another (e.g., arrestin-mediated signaling), although the molecular mechanisms responsible for these differences are unknown (Urban et al., 2007). The authors suggest that such biased ligands trigger distinct changes in the cytoplasmic half of the receptor, leading to a receptor with drastically limited dynamic properties. Biased ligands appear then to facilitate the interaction with certain signaling partners with higher probability (see Figure 1). The model proposed, based on these results and those of others (Liu et al., 2012), is that biased ligands that preferentially stabilize noncanonical arrestin-ergic signaling have an overall lower effect on receptor conformations than unbiased ligands.

The proposed model does not yet clarify how interactions with different cellular partners (e.g., G proteins, arrestins and so on) affect the observed dynamic structural ensemble. Certainly, GPCRs can interact with G proteins independently of

agonist and thereby allosterically modulate GPCR structure (Yan et al., 2008). Based on the results presented here, one can speculate that the interactions with G proteins shift the conformational ensembles of the extracellular half of the receptor to those favoring agonists. Further study is needed to determine the differences between the dynamic ensembles correlated with different biased ligands and to clarify how different cellular partners change GPCR dynamics. Additionally, comparing high-resolution structures (e.g., via X-ray crystallography) of arrestin-biased versus full agonists will help to explicate how different ligands can induce drastically different patterns of intracellular signaling.

Because it is well accepted that GPCRs—like all proteins—continuously vibrate, their dynamic nature allows them to execute the myriad roles they have in biology (Kenakin, 2002). Modifying the dynamic range of a GPCR and the conformational space that it can subsequently explore results in changes in its activity—in this case, the activation of a specific signaling pathway. Illuminating the dynamic nature of GPCRs, as done here, offers a key to understanding the molecular mechanisms of biased and nonbiased signaling. As shown here (Nygaard et al., 2013), the ability to follow the chemical environments of strategically placed methionines within a prototypical GPCR combined with incredibly long molecular dynamics simulations now places the dynamic behavior of the receptor into a structural context and provides initial glimpses of the protein dynamics that lead to signaling.

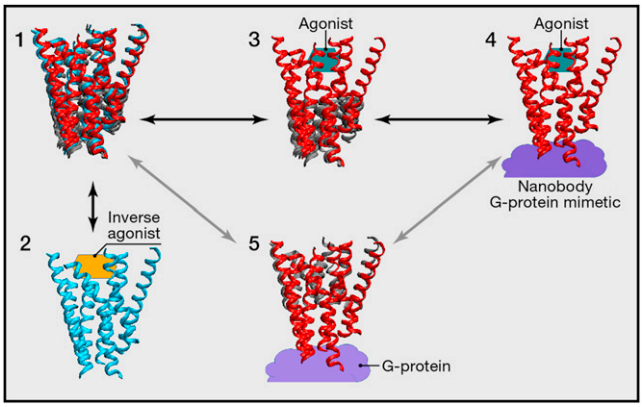

Figure 1. GPCR Conformational Ensembles Illuminated

(1) The ligand-free receptor samples a large spectrum of available conformations ranging from active (red) to inactive (blue). (2) Inverse agonists stabilize the inactive ensembles of conformations (blue). (3) Extracellular agonist binding promotes an enrichment of "signaling" ensembles (gray) at the cytoplasmic end of the receptor. Nygaard et al. suggest that "biased" agonists can both promote and inhibit conformational ensembles, thus promoting the interactions of the receptor with different cellular partners. (4) The signaling ensemble favored by the agonist is further stabilized by the interaction with the G protein mimetic NB80. As a consequence, binding of both agonist and G protein analog are necessary for achieving the fully active conformation. (5) We also illustrate a proposed effect that interaction of a GPCR with a G protein could have on the agonist binding properties of the receptor; presumably, this G protein complex would shift the structural space of the extracellular half of the receptor toward states that favor agonist binding.

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